MATHEMATICAL MODEL OF BIOTECHNOLOGY PROCESS TO PRODUCE A RECOMBINANT PROTEIN

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Introduction

Predictive modeling is a tool that can be applied to biotechnology. The models are used to describe the behavior of microorganisms varying the physical and chemical conditions such as temperature, pH and activity of the aqueous solution. These models allow to have the prediction of the microbial productivity, the control of critical phase and the optimization of the production process. Bacterial growth often shows different phases: a latency phase, a growth phase and an asymptotic phase. For the fermentations are considered batch, continuous and feed-bach operations.

The aim of this research is to devObjectives

elop a mathematical model to describe the production of human antibody fragments of small size as ScFv, Fab, F(ab'), through fermentation of Escherichia coli BW25113 (ara) [1, 2, 3].

Materials and method

The fermentations are conducted in a fermenter (Chemap Ag) with a mechanical agitation. The entire phase of fermentation is monitored on-line using a data acquisition system MFCS/WIN. A kinetic and stochiometric models are developed. The stochiometric model describes the biological process of biomass growth. The kinetic analysis of experimental data about fermentation of E. coli is carried out for batch and fed-batch phase for the production process. The batch analysis is described by material balances of substrate and biomass with Monod and Pirt equations. The fed-batch phase is modelled using the material balances on biomass, substrate, product and analyzing the variation on volume during the time. Runge and Kutta algorithm is used to resolve the system equations.

Figure 1. Biomass monitor used in the experimental tests

Figure 2. Electrolab FerMac 368 Gas Analyser used in the experimental tests

References: 1.Jenzsch M, Simutis R, Lüebbert A (2006) Generic Model Control of the Specific Growth Rate in Recombinant Escherichia coli Cultivation. J. Biotechnol. 122:483-493. 2. Rocha I, Ferreira EC, (2004) Yield and Kinetic Parameters Estimation and Model Reduction in a Recombinant E. coli Fermentation. In: European symposium on computer-aided process engineering, Lisbon. Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal. 3. Faulkner E, Barret M, Okor S, Kieran P, Casey E, Paradisi F, Engel P and Glennon B (2006) Use of Fed-Batch Cultivation for Achieving High Cell Densities for the Pilot Scale Production of a Recombinant Protein (Phenylalanine Dehydrogenase) in E. coli., Biotechnol. Progr., 22:889-897.

Results

Result show that the equation that describe the growth of biomass is: $C_6H_{12}O_6 + 3.56O_2 + 0.52ONH_3 \rightarrow 2.13CH_{1,92}O_{0,3}N_{0,24} + 3,87CO_2 + 4,74H_2O$. For the Monod and Pirt law the following parameters are found by regression of experimental data during the batch phase: μ_{max} is 0.55 h^{-1}, Ks is 0.10 g/L, Yx/s is 0.35. The kinetics parameters that describe the fed-batch phase are the following: μ_{max} is 0.24 h-1, Ks is 1.5 g/L, Yx/s is 0.34, m is 0.02, α is 0.00043, β is 0.00007, Yp/s is 0.00084.

A sensitivity analysis is carried out to verify the efficiency of the mathematical model, varying the values of parameters about ±10%: evident variations are not present so the model is robust and stable.

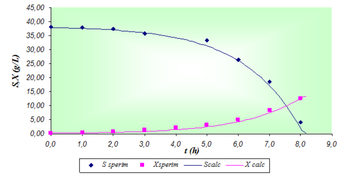

Figure 3. Consumed substrate and microbe growth during the time

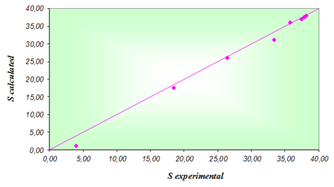

Figure 4. Calculated consumed substrate versus experimental consumed substrate

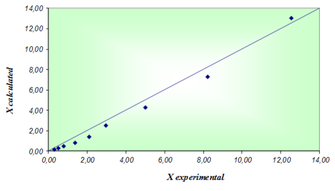

Figure 5. Calculated growth of biomass versus experimental growth of biomass

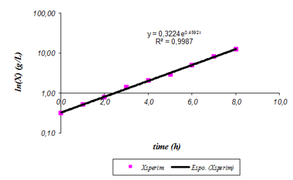

Figure 6. Dry weight (in logarithmic scale) over time

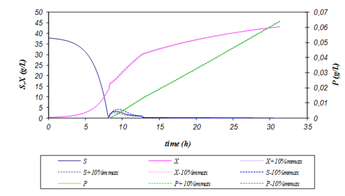

Figure 7. Sensitivity analysis of μ_{max}

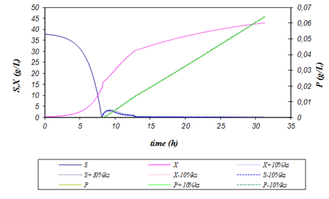

Figure 8. Sensitivity analysis of Ks

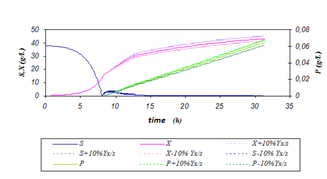

Figure 9. Sensitivity analysis of Yx/s

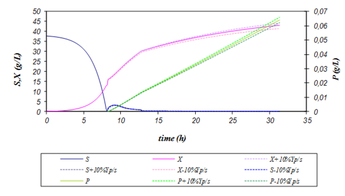

Figure 10. Sensitivity analysis of Yp/s

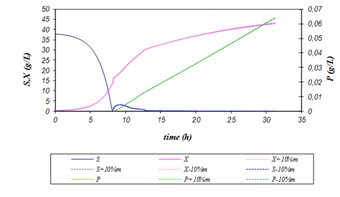

Figure 11. Sensitivity analysis of m

Conclusions

The realized mathematical models can be used to optimize the pilot plant and for the planning of the laboratory tests.